2016/05/19

• Ruijie Tang • May 19, 2016 @12:13 PM EDT

2016/05/19

1. Picking colonies:

   TetR + GFP: three colonies

   TetR + Prom: one colonies

   Colicin: two colonies

   Others: none

2. We prepared LB tubes and put it into the autoclave

3. We digested the Registry Linearization Plasmid

   All four for 5ul with 15ul of master mix in each tube

4. We put the tubes into the dry incubator and labeled "Digested Backbone 2016/05/20"

2016/05/31

• Lydia Goff • Jun 01, 2016 @09:06 AM EDT

5/31/16

1. Performed competent cell procedure (4 tubes resulted)

2. Transformation procedure for competent cells (6 tubes resulted)

3. Made two 300 ml containers of agar for new Amp and Chl plates.

4. Plated

2016/06/01

• Lydia Goff • Jun 01, 2016 @11:40 AM EDT

6-1-16

1. Picked colonies. All plates had colonies so three LB tubes were made from each and placed in shaking incubator. 2-3 looked like there might be contamination.

2. Made plates. 12 Amp. 10 Chl.

2016/06/02

• Lydia Goff • Jun 02, 2016 @12:42 PM EDT

6-2-16

1. Plasma prep procedure

   a. 17 tubes out of 18 (3 from each plate) grew. 1 pBAD GFP tube did not grow.

2. Competent cell procedure. 6 tubes filled with E. Coli from fridge.

2016-06-03

• Lydia Goff • Jun 06, 2016 @09:24 AM EDT

6-3-16

1. Digest procedure with 17 plasmid prep tubes

2. Two gels made

3. Right gel: pBAD RBS, pBAD RBS, pBAD RBS, pBAD tetR, pBAD tetR, pBAD tetR

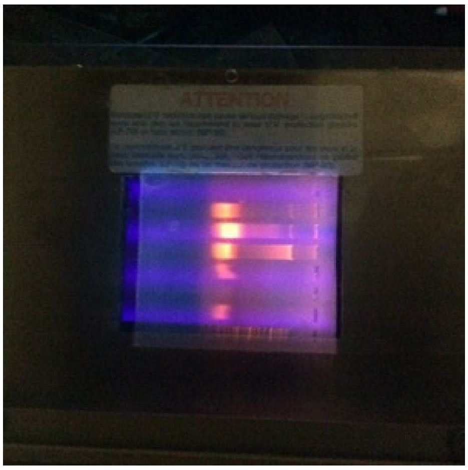
Left Gel top row: tetR prom, lysis, lysis, lysis, pBAD GFP, pBAD GFP

Red/bottom row: tetR prom, tetR prom, colicin, colicin, colicin

• Lydia Goff • Jun 03, 2016 @11:27 AM EDT

6-3-16_Gel_left_two_rows.jpg(821.5 KB) - download

• Lydia Goff • Jun 03, 2016 @11:27 AM EDT



6-3-16_Gel_right_single_row.jpg(688.3 KB) - download

2016-06-06

• Lydia Goff • Jun 20, 2016 @10:08 AM EDT

6-6-16

1) Looked at streaks.

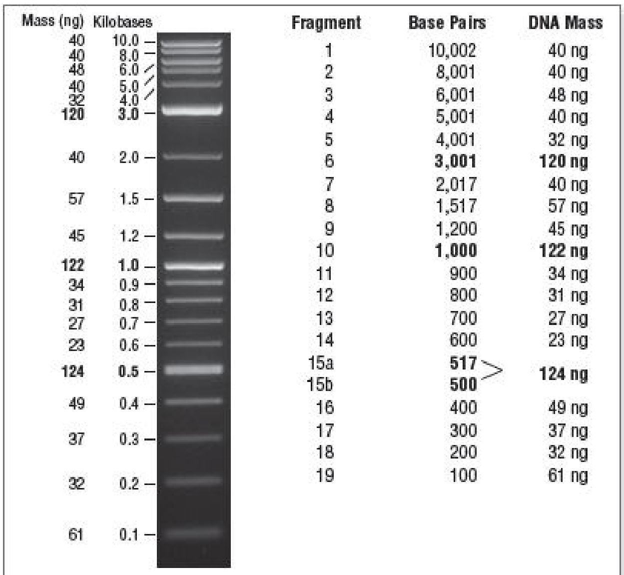
2) Digested plasmids again

3) Ran gel

In gel 1) ladder, colicin (3), pBAD tetR (3)

In gel 2) ladder, tetR prom (3), pBAD RBS (3)

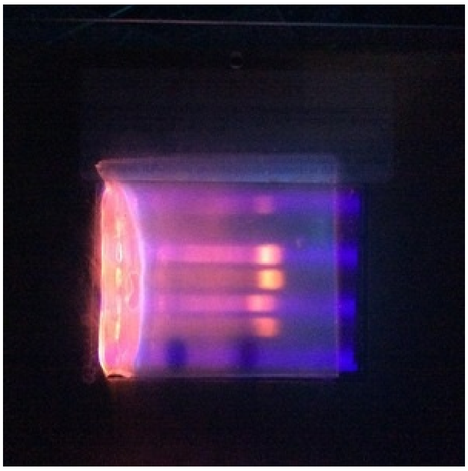
In gel 3) ladder, pBAD GFP (2), lysis (3)



Fragment	Base Pairs	DNA Mass
1	10,002	40 ng
2	8,001	40 ng
3	6,001	48 ng
4	5,001	40 ng
5	4,001	32 ng
6	**3,001**	**120 ng**
7	2,017	40 ng
8	1,517	57 ng
9	1,200	45 ng
10	**1,000**	**122 ng**
11	900	34 ng
12	800	31 ng
13	700	27 ng
14	600	23 ng
15a	**517**	**124 ng**
15b	**500**	
16	400	49 ng
17	300	37 ng
18	200	32 ng
19	100	61 ng

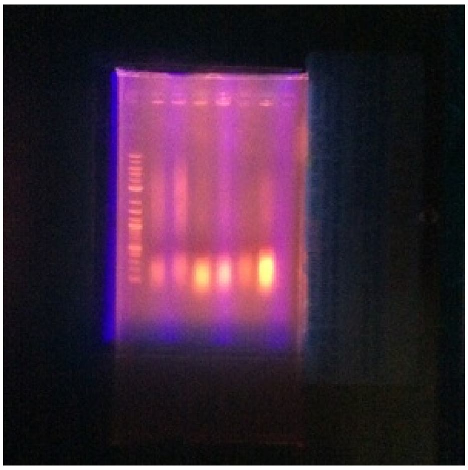
Quick-Load 2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 µg/lane.

• Lydia Goff • Jun 20, 2016 @10:07 AM EDT



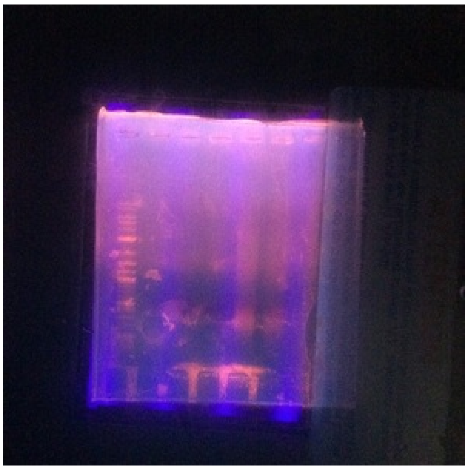
6-6-16_Gel_well_1.JPG(635.1 KB) - download Gel 1-colicin and pBAD tetR Ladder did not run so it cannot be read. Probably rerun.

▪ Lydia Goff ▪ Jun 20, 2016 @10:07 AM EDT



6-6-16_Gel_well_2.jpg(788.5 KB) - download Ge1 2 Wells 1-3, tetR prom, should be 127 base pairs. Appears to be between 100-400. We will move on with this piece since it seems right. Wells 4-6,pBAD RBS, should be 203 base pairs. Appears to be between 200-400. We will move on with this piece since it seems correct.

▪ Lydia Goff ▪ Jun 20, 2016 @10:07 AM EDT



6-6-16_Gel_well_3.JPG(829.7 KB) - download Gel 3- pBAD GFP and lysis Rerun these since gel messed up and can't be read.

2016-06-07

▪ Lydia Goff ▪ Jun 08, 2016 @09:14 AM EDT

6-7-16

1.Transformed InterLab measurement kit (Device 1, Device 2, Device 3, Negative Control, Positive Control)

2. Began research on arabinose induction

2016-06-08

▪ Lydia Goff ▪ Jun 20, 2016 @09:07 AM EDT

8-6-16

1. Transformations from measurement kit have no growth.

2. Made Chl plates

3. Arabinose induction concentrations

4. Ligation of Tet R Prom GFP with Chlor and Tet R Prom GFP with Amp.

2016-06-20

• Lydia Goff • Jun 20, 2016 @12:31 PM EDT

1. Transformation procedure for ligations tetR prom GFP with Chlor and tetR prom GFP with amp. Put in incubator.
2. Analyzed gels from 2016-06-06. Decided to rerun everything in gels 1 and 3 since those could not be read. 2 seemed to fit so we will continue on with those.

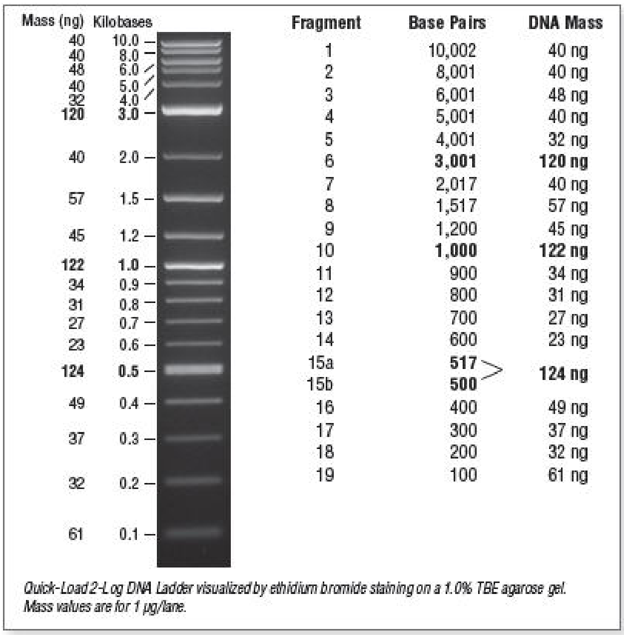
2016-06-21

• Lydia Goff • Jun 21, 2016 @12:35 PM EDT

1. Transformed measurement kit again in hopes that there will be growth this time and plated them.
2. Plated tetR prom GFP (1 Amp and 1 Chlor) transformations from yesterday.
3. Made digests from the plasmid preps from gels 1 and 3 (6-6-16) that did not work.
4. Ran 2 gels (10 wells) from digests

   Gel 1. pBAD GFP, lysis, lysis, pBAd GFP, colicin

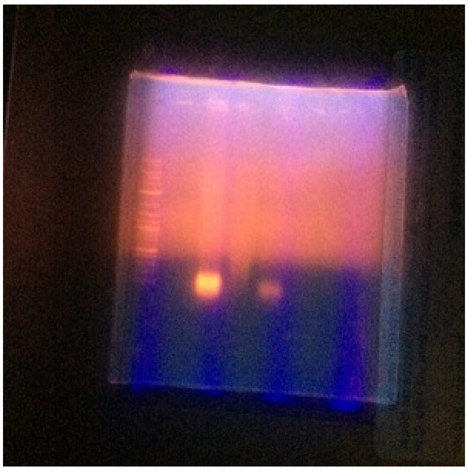
   Gel 2. pBAD tetR, colicin, pBAD tetR, lysis, pBAD tetR



Mass (ng)	Kilobases	Fragment	Base Pairs	DNA Mass
40	10.0	1	10,002	40 ng
40	8.0	2	8,001	40 ng
48	6.0	3	6,001	48 ng
40	5.0	4	5,001	40 ng
32	4.0	5	4,001	32 ng
120	**3.0**	6	**3,001**	**120 ng**
40	2.0	7	2,017	40 ng
57	1.5	8	1,517	57 ng
45	1.2	9	1,200	45 ng
122	**1.0**	10	**1,000**	**122 ng**
34	0.9	11	900	34 ng
31	0.8	12	800	31 ng
27	0.7	13	700	27 ng
23	0.6	14	600	23 ng
124	**0.5**	15a	**517**	**124 ng**
		15b	**500**	
49	0.4	16	400	49 ng
37	0.3	17	300	37 ng
32	0.2	18	200	32 ng
61	0.1	19	100	61 ng

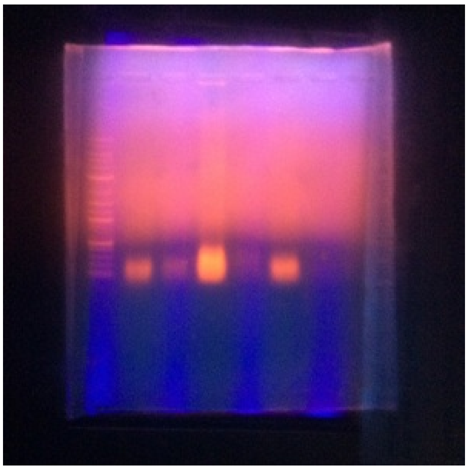
Quick-Load 2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 µg/lane.

• Lydia Goff • Jun 21, 2016 @12:34 PM EDT



IMG_0075.JPG(927.7 KB) - download Gel 1 pBAD GFP, lysis, lysis, pBAd GFP, colicin

• Lydia Goff • Jun 21, 2016 @12:35 PM EDT



IMG_0078.JPG(771.3 KB) - download Gel 2 pBAD tetR, colicin, pBAD tetR, lysis, pBAD tetR

2016-06-22

• Lydia Goff • Jun 22, 2016 @11:41 AM EDT

1. Made digests from pBAD GFP, pBAD tetR, tetR prom RBS, and pBAD RBS

2. Ligation procedure for digest with Amp instead of Chl because out of Chl backbone.

3. Project description finished and being reviewed by other leaders and advisor.

2016-06-23

• William Henry • Jun 23, 2016 @11:27 AM EDT

1. Plasmid prepped interlab measurement negative control colonies and ran a gel. Gel came out with no bands for anything, not even ladder. Only the ladder well fluoresced.

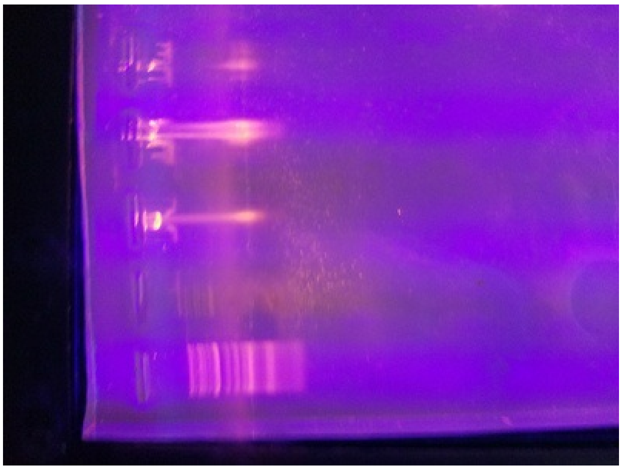
2. Made competent cells and transformed yesterday's ligations.

2016-06-27

• William Henry • Jun 27, 2016 @11:17 AM EDT

1. Plasmid Prepped and ran a gel of Tet R Prom GFP 1-4.
2. Created More LB tubes and the like.
3. Planning an e-mail to Vernier about their SpectroVis Plus settings for GFP

• William Henry • Jun 28, 2016 @09:06 AM EDT



Gel.jpg(3.5 MB) - download

• William Henry • Jun 28, 2016 @09:07 AM EDT

The wells are as follows

1. Ladder
2. Tet R Prom GFP 1
3. Tet R Prom GFP 2
4. Tet R Prom GFP 3
5. Tet R Prom GFP 4

2016-06-28

• William Henry • Jun 28, 2016 @12:10 PM EDT

1. Reviewed video sent by Vernier about the SpecVis.
2. Continued work on project summary.
3. made competent cells and started a transformation on the 5 strands of DNA. Ended on step 15
4. Ran absorbance and florescence tests for Tet R Prom GFP 3 and 4 from new cultures grown from yesterday.
    1. For the OD 600.3:
        1. PBS: 0.00
        2. Tet R Prom GFP 3: 0.511
        3. Tet R Prom GFP 4: 0.615
    2. For the Florescence at 500nm
        1. PBS: 0.028
        2. Tet R Prom GFP 3: 0.563
        3. Tet R Prom GFP 4: 0.721
    3. For the Florescence at 405nm
        1. PBS: 0.016
        2. Tet R Prom GFP 3: 0.855
        3. Tet R Prom GFP 4: 0.974

2016-06-30

• Lydia Goff • Jul 01, 2016 @12:00 PM EDT

1. Plasmid preps, tetR prom RBs (4), pBAD GFP (4), pBAD tetR (4)

2. Started growth curves but tech issues prevented completion. (Resume on 7th)

2016-07-01

• Lydia Goff • Jul 01, 2016 @11:49 AM EDT

1. Digests of yesterday's plasmids

2. Made plasmids from colicin cultures (A-1, A-2, A-3, C-1, C-2, C-3)

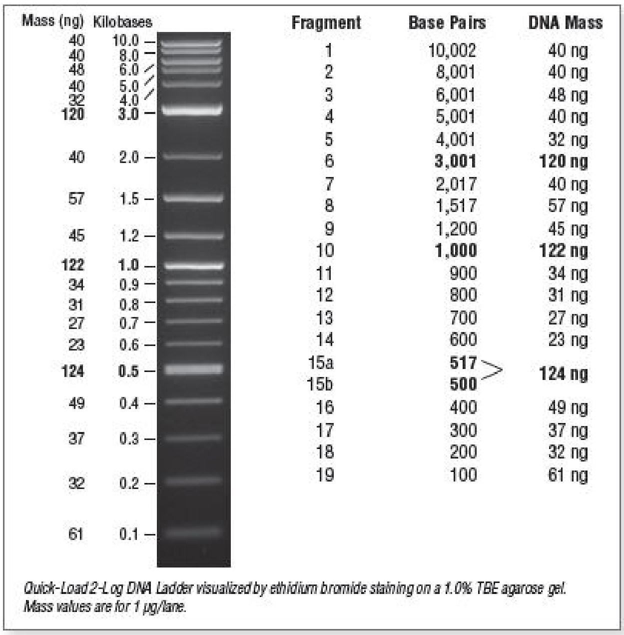
3. Streaked plate with TetR prom GFP 3 and 4 (leaving on table over weekend)

4. Ran digests in gel

Gel 1: tetR prom RBS 1-4, pBAD GFP 1-2

Gel 2: pBAD GFP 3-4, pBAD tetR 1-4

5. Dumping tetR prom RBS; rerunning everything else. Hopeful about tetR prom RBS 1 and 4.



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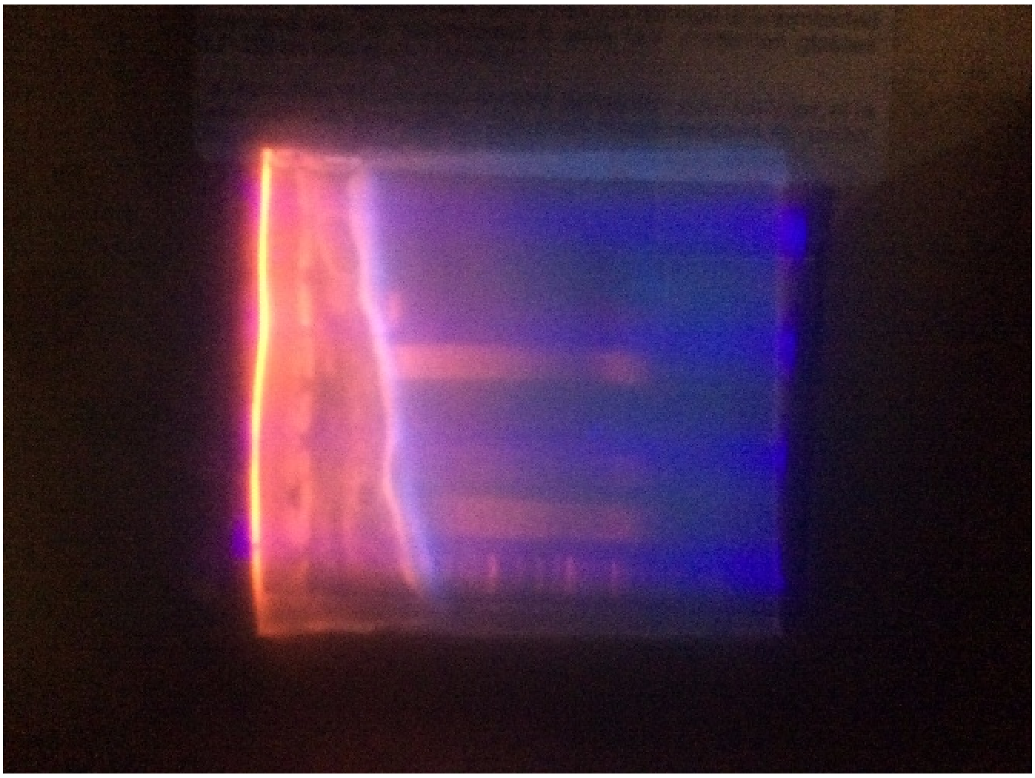
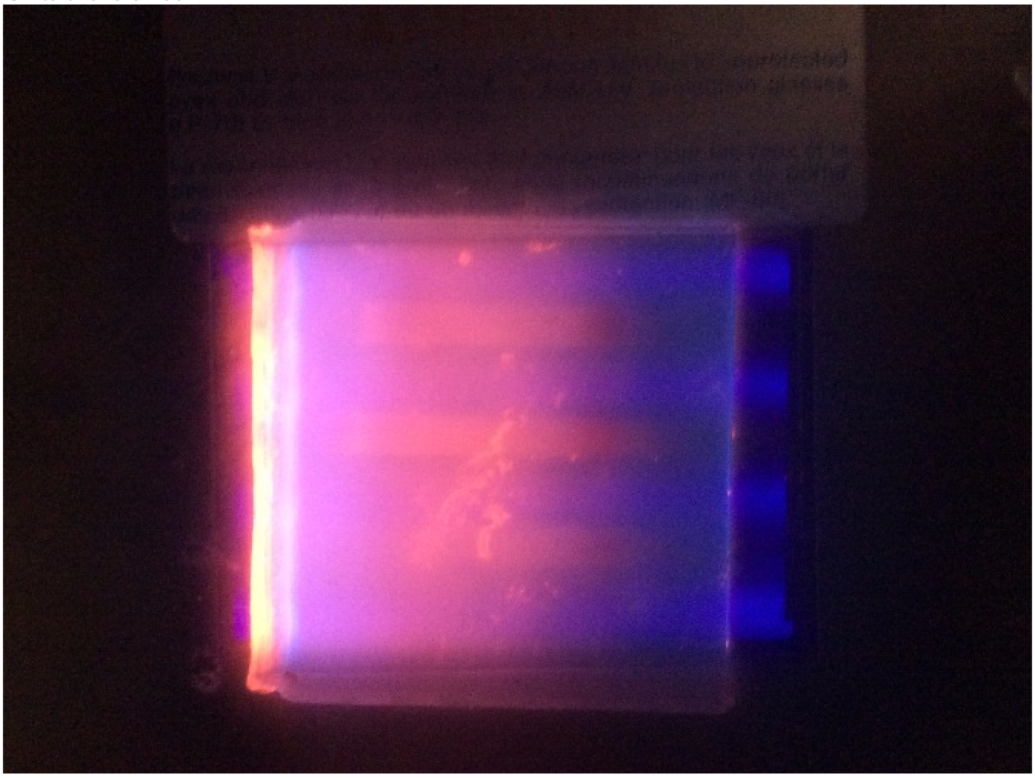
Quick-Load 2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel. Mass values are for 1 µg/lane.

GEL 1



GEL 2



2016-07-06

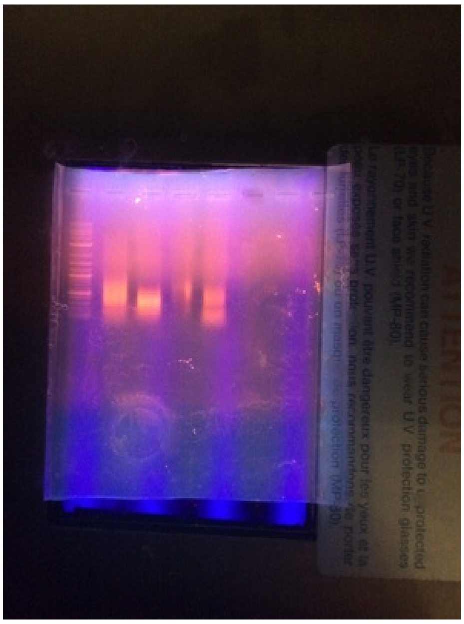
• Lydia Goff • Jul 06, 2016 @11:08 AM EDT

1. Digests from Colicin A 1-3, Colicin C 1-3, and TetR prom GFP 3 and 4 (8 total)
2. Working on growth curve again. Freezing samples after taking 1 ml every 30 minutes, spinning down, and decanting.
3. Ran gels:

   Gel 1: Colicin A-1, A-2, A-3, and Colicin C-1

   Gel 2: Colicin C-2, C-3, Tet R prom GFP 3, and TetR prom GFP 4

• Lydia Goff • Jul 06, 2016 @12:50 PM EDT



IMG_0102.JPG(766.2 KB) - download

▪ Lydia Goff ▪ Jul 06, 2016 @12:35 PM EDT

IMG_0104.JPG(1016.2 KB) - download

2016-07-07

▪ Lydia Goff ▪ Jul 07, 2016 @11:38 AM EDT

1. Plasmid preps for lysis A-F and TetR prom A-F
2. Growth curves from yesterday done through number 15 and new set started labeled B. Will run both of these tomorrow.
3. Picked colonies from TetR prom GFP 3 and 4. (5 each)
4. Streaked plates for lysis D, E, and F anf TetR prom A, C, and E.

2016-07-08

• Lydia Goff • Jul 08, 2016 @01:20 PM EDT

1. Made more 1xPBS

2. Made LB plates

3. Digests for plasmid preps from yesterday

4. Ran growth curves (1-4 had original supernatant dumped before I learned they had not been resuspended. Resuspended with new PBS.)

NOTE: Because of labeling issues that caused us to believe 1 and 7 cuvettes may have been mixed up, we changed what was labeled as 7 to 1 and vice versa as the absorbance rates made more sense that way.

Absorbance vs. Concentration: Growth Curve A

0 - 0

1 - 0.018

2 - 0.154

3 - 0.077

4 - 1.494

5 - 0.993

6 - 1.155

7 - 1.329

8 - 0.265

9 - lost

10 - 0.093

11 - 0.176

12 - 0.158

13 - 0.272

14 - 0.416

15 - 0.479

Growth Curve B

0 - 0

1 - 1.406

2 - 0.009

3 - 0

4 - 0.018

5 - 0

6 - 0.059

7 - 1.047

8 - 0.129

9 - 1.151

10 - 0.186

11 - 0.836

12 - 0.448

13 - 0.461

14 - 0.497

2016-07-11

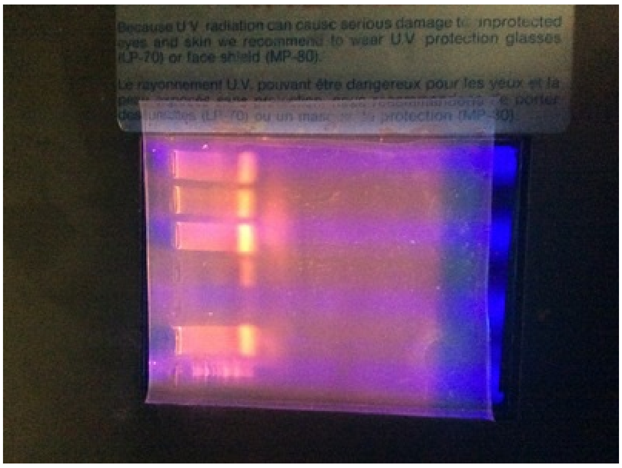
• William Henry • Jul 11, 2016 @11:35 AM EDT

1. Ran a gel of TetR Prom and Lysis
2. Plasmid Prepped what we ran in the gel
3. Created a 4 mL E. coli cultue
4. Created 4 4 mL cultures of TetR + GFP 3,4

• William Henry • Jul 11, 2016 @10:38 AM EDT

IMG_0109.JPG(957.1 KB) - download

• William Henry • Jul 11, 2016 @10:38 AM EDT



IMG_0107.JPG(966.4 KB) - download

2016-07-12

▪ Lydia Goff ▪ Jul 13, 2016 @09:09 AM EDT

1. a) TetR GFP 3 A-D and 4 A-D (8 tubes) cultures spun down and decanted. Resuspended in 4 ml of PBS.

OD 600: Tet R GFP 3A: 0.597

Tet R GFP 3B: 0.317

Tet R GFP 3C: 0.564

Tet RGFP 3D: 0.630

Tet R GFP 4A: 0.625

Tet RGFP 4B: 0.375

Tet RGFP 4C: 0.374

Tet RGFP 4D: 0.442

b)Picked 8 more colonies from TetR GFP plate (4 from 3 and 4 from 4)

2. Digests from plasmid preps.

3. Growth curve (every 30 minutes)

0-0

1-0

2-0.026

3-0.058

4-0.126

5-0.268

6-0.411

7-0.564

4. Gels from digests

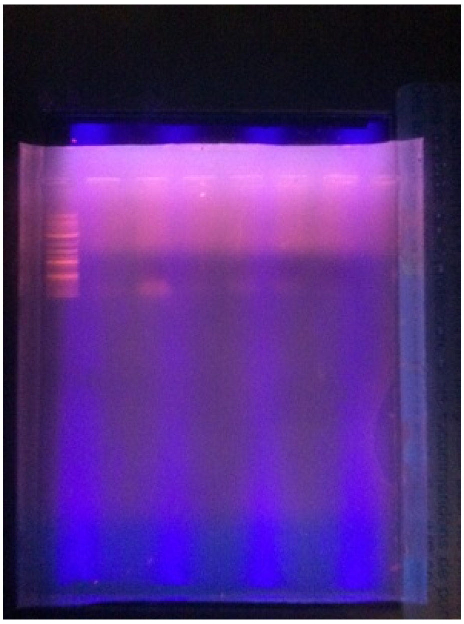
Gel 1: tetR Prom F2, lysis F1, lysis F2, tetR Prom A2, tetR Prom A1, lysis E1

Gel 2: tetR Prom E1, lysis D1, lysis E2, tetR Prom C2, tetR Prom C1, lysis D2

▪ Lydia Goff ▪ Jul 12, 2016 @12:00 PM EDT

IMG_0111.JPG(1.1 MB) - download Gel 1

• Lydia Goff • Jul 12, 2016 @12:00 PM EDT



IMG_0113.JPG(1 MB) - download Gel 2

2016-07-13

• Lydia Goff • Jul 13, 2016 @12:35 PM EDT

1. Spun down TetR GFP 3 A-D and TetR GFP 4 A-D colonies, decanted, and resuspended in 4 ml PBS

OD 600:

3A-0.590

3B-0.465

3C-0.499

3D-0.571

4A-0.412

4B-0.352

4C-0.462

4D-0.393

Fluorescence (diluted to 1/10)

3A-0.278

3B-0.110

3C-0.179

3D-0.165

4A-0.345

4B-0.394

4C-0.287

4D-0.180

2. Plasmid preps from pBAD GFP, then digested.
3. Fresh cultures made from pBAD GFP A and B and pBAD tetR 1-4.
4. Ran gel with pBAD GFP A and B digest

2016-07-14

• Lydia Goff • Jul 14, 2016 @11:55 AM EDT

1. Plasmid preps pBAD GFP A and pBAD GFP B

2. Spun down pBAD tetR 1-4 and resuspended in 4ml of PBS.

OD 600

pBAd tetR 1- 0.813

pBAD tetR 2- 0.238

pBAD tetR 3-0.534

pBAD tetR 4-0.751

Fluorescence (diluted 1:10)

pBAD tetR 1- 0.260

pBAD tetR 2- 0.168

pBAD tetR 3- 0.222

pBAD tetR 4- 0.220

3. Ligation for pBAD tetR chl, pBAD tetR amp, pBAD GFP chl, and pBAD GFP amp

2016-07-15

• Lydia Goff • Jul 15, 2016 @10:54 AM EDT

1. Made competent cells. Transformed yesterday's ligations.

2. Growth curves: one directly from culture and one resuspended in PBS to test variables.

2016-07-26

• Lydia Goff • Jul 27, 2016 @09:05 AM EDT

Started pBAD GFP cultures

2016-07-27

• Lydia Goff • Jul 27, 2016 @09:05 AM EDT

2016-08-03

• Lydia Goff • Aug 03, 2016 @09:39 AM EDT

Last few days:

1. Made new ligations for pBAD GFP and pBAD tetR both in chl

2. Transformations

3. plated transformations on chl

4. pBAD GFP plate has glowing colonies under UV light and pBAD tetR does not!!! Progress. Picked four colonies from each and placed in shaking incubator.

2016-08-04

• William Henry • Aug 04, 2016 @10:51 AM EDT

Completed plasmid prep of pbad GFP A-D and pbad tetR A-D.

• William Henry • Aug 04, 2016 @11:39 AM EDT

We also digested the plasmid preps completed today

2016-08-05

• Shetal Patel • Aug 05, 2016 @10:56 AM EDT

1. Running digests from yesterday

2. Made new rack of LB tubes

3. Repeating plasmid preps from yesterday (pBAD tetR A-D and pBAD GFP A-D, 8 total)

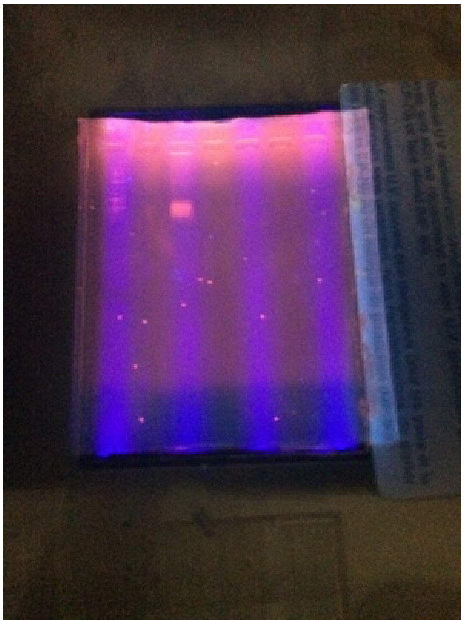
4. Gel 1: pBAD GFP A-D

Gel 2: pBAD tetR A, C, D (B is MIA) (7 total)

• Shetal Patel • Aug 05, 2016 @11:50 AM EDT

IMG_0119.JPG(1.2 MB) - download Gel 1

• Shetal Patel • Aug 05, 2016 @11:50 AM EDT



IMG_0122.JPG(1.2 MB) - download Gel 2

2016-08-08

• Lydia Goff • Aug 08, 2016 @10:57 AM EDT

• Lydia Goff • Aug 08, 2016 @12:00 PM EDT

1. Digests for pBAD GFP A-D and pBAD tetR A-D

2. pBAD tetR A-D in Gel 1

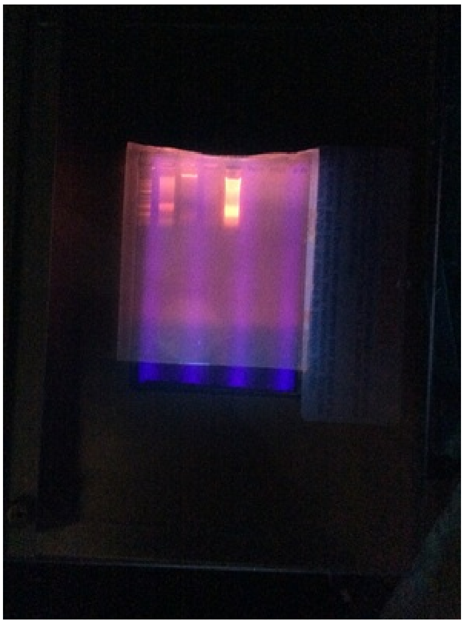
pBAD GFP A-D in Gel 2

3. Made fresh competent cells

4. Transformation for lysis and colicin

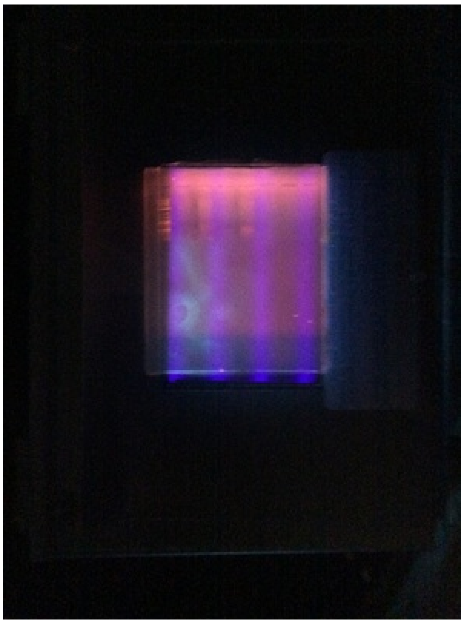
5. pBAD tetR A and D are keepers. Will submit to competition.

• Lydia Goff • Aug 08, 2016 @11:59 AM EDT



IMG_0124.JPG(946.6 KB) - download gel 1- tetR

• Lydia Goff • Aug 08, 2016 @11:59 AM EDT



IMG_0125.JPG(906.2 KB) - download Gel 2-GFP

2016-08-09

• Lydia Goff • Aug 09, 2016 @09:44 AM EDT

1. Picked 3 colonies from lysis (chl and amp) and colicin (chl and amp) plates (12 total)

NOTE: Lysis and colicin were grown on chl and amp plates because their antibiotic resistance was not recorded on the tubes. Better colonies were on the amp plates for both but there were still colonies on the chl plates.

2. Competent cells

3. Plasmid preps of pBAD tetR A and D and tetR prom GFP 3D and 4D

2016-08-10

• Lydia Goff • Aug 10, 2016 @11:53 AM EDT

1. Plasmid preps for colicin and lysis samples (Lysis B had odd pellet)

2. Picked two colonies from pBAD GFP A-D plates from yesterday. (8 total; one from lawn and one from colony for each)

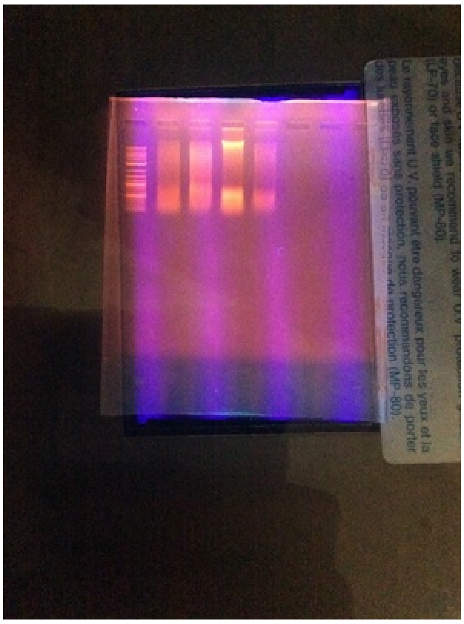
3. Made fresh competent cells

4. Transformed pBAD tetR A and D and TetR prom GFP 3D and 4D and plated in Kan

5. Digest of pBAD GFP A-D plasmid preps from yesterday

6. Ran digests in gel. Keeping pBAD GFP B and C. B is best. A and D will be dumped.

• Lydia Goff • Aug 10, 2016 @11:52 AM EDT



IMG_0126.JPG(1.2 MB) - download

2016-08-11

• Lydia Goff • Aug 11, 2016 @11:27 AM EDT

1. Digests of colicin and lysis samples

2. Starting functional tests from pBAD GFP B1, B2, C1, and C2 (1 represents colonies picked from lawn and 2 represents colonies picked from clusters on plates)

6 tubes per sample; 2 each of 0%, 0.1%, and 1% arabinose concentration; 3 of each sample will be tested in a few hours (marked with S) and the remaining 3 will be tested tomorrow after growing overnight (marked with L)

24 tubes total

3. Made more LB tubes

4. Plates from yesterday wrapped and stored in fridge

5. Ran gels

Gel 1: colicin A chl, colicin A amp, colicin B chl, colicin C chl, colicin C chl, colicin C amp

Gel 2: lysis A chl, lysis B chl, lysis B amp, lysis C amp

• Lydia Goff • Aug 12, 2016 @11:37 AM EDT

IMG_0130.JPG(1.1 MB) - download

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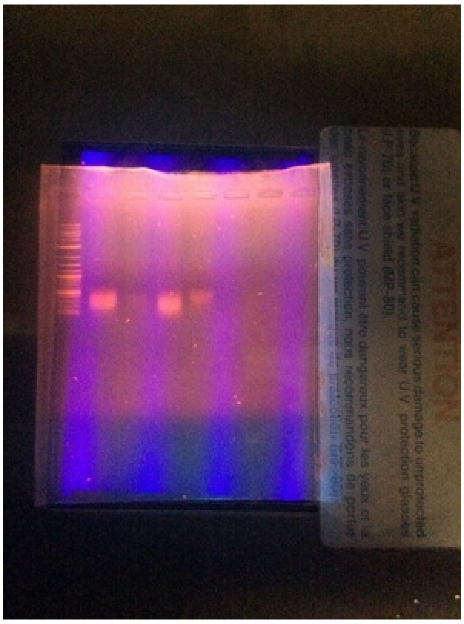

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2016-08-12

▪ Lydia Goff ▪ Aug 12, 2016 @11:36 AM EDT

1. OD 600 and fluor 405 nm for pBAD GFP B1, B2, C1, and C2 (the ones meant to be examined late afternoon were not so instead of afternoon and overnights we did LB and resuspended in PBS tests)

2. LB: OD 600; flour 405nm

B1 0%: 0.482; 0.022

B1 0.1%: 0.457: 0.022

B1 1%: 0.341; 0.021

B2 0%: 0.477; 0.022

B2 0.1%: 0.727; 0.023

B2 1%: 0.479; 0.022

C1 0%: 0.411; 0.022

C1 0.1%: 0.098 (some was spilled); 0.022

C1 1%: 0.306; 0.022

C2 0%: 0.578; 0.022

C2 0.1%: 0.697; 0.023

C2 1%: 0.609; 0.023

3.

PBS: OD 600; fluor 405 nm

B1 0%: 0.273; 0.018

B1 0.1%: 0.391; 0.018

B1 1%: 0.358; 0.019

B2 0%: 0.527; 0.020

B2 0.1%: 0.533; 0.019

B2 1%: 0.628; 0.020

C1 0%: 0.211; 0.018

C1 0.1%: 0.316; 0.018

C1 1%: 0.269; 0.018

C2 0%: 0.556; 0.020

C2 0.1%: 0.513; 0.019

C2 1%: 0.517; 0.020

6/2/16

▪ Lydia Goff ▪ Jun 02, 2016 @12:44 PM EDT

1. Started transformation but tubes appeared empty. 5 tubes with 50 ul of competent cells are now in fridge.

6/7/16

▪ Lydia Goff ▪ Jun 20, 2016 @09:09 AM EDT

1. Transformed InterLab Measurement

6/8/16

▪ Lydia Goff ▪ Jun 20, 2016 @09:10 AM EDT

1. No growth on transformations

6/21/16

▪ Lydia Goff ▪ Jun 21, 2016 @11:49 AM EDT

1. Transformed positive and negative control and test devices 1,2, and 3 again.
2. Plated transformations.

6/22/16

▪ Lydia Goff ▪ Jun 22, 2016 @11:49 AM EDT

1. Checked transformations. Two colonies on negative control but nothing on the rest. Growing negative control in shaking incubator.
2. Unless there are colonies tomorrow, will be done with measurement kit.

7/18/16

▪ William Henry ▪ Jul 18, 2016 @11:41 AM EDT

1. Created competent cells for previous week's ligation
2. Started a digest for pBAD TetR1-4 *pBAD TetR 4 may not have any DNA in it because mistakes were made